*Zootaxa* 3450: 12–22 (2012)
www.mapress.com/zootaxa/


ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

**Article**

urn:lsid:zoobank.org:pub:1B1F1BBC-BAB5-48F8-93CE-F10D831A71FC

# On the diversity of the genus *Pisione* (Polychaeta, Pisionidae) along the Portuguese continental shelf, with a key to European species

ROBERTO MARTINS[1], GUILLERMO SAN MARTÍN[2], ANA MARIA RODRIGUES[1] & VICTOR QUINTINO[1,3]

[1]*Departamento de Biologia & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal*
[2]*Departamento de Biologia, Facultad de Ciencias, Calle Darwin 2, Universidad Autónoma de Madrid, 28049 Madrid, España*
[3]*Corresponding author. E-mail: victor.quintino@ua.pt*

## Abstract

This work details the diversity and distribution of the genus *Pisione* Grube, 1857, Family Pisionidae Southern, 1914, on the Portuguese continental shelf. The study reports the first records for this region of the species *P. guanche*, *P. inkoi* and *P. parapari,* where previously *P. remota* was the only species reported. A detailed morphological study of the four species is presented, with a discussion of habitat preferences and biogeographic issues related to their distributional ranges. A total of 692 specimens were recorded at 48 sites. The four species coexist, with *P. remota* and *P. parapari* being the most abundant. A multivariate analysis based on morphological descriptors of 75 specimens showed a good separation of the four species. *Pisione guanche* and *P. inkoi* are characterized by a protruding notoacicula, longer in *P. inkoi*. These two species can be differentiated by the proportional length of the dorsal cirrus on parapodia 2 compared to parapodia 3, much longer in *P. guanche*, and by the number of distal teeth in the supra-acicular simple chaetae, bidentate in *P. guanche* and unidentate in *P. inkoi*. Of the four species, *P. remota* is the only one with an infra-acicular simple chaeta. The smallest intra-specific variability was found in *P. parapari* and the highest in *P. guanche*. The variability within species was much lower than the inter-specific variability which validated the four species of *Pisione* occurring in the Iberian Peninsula. This work set the meridional limit of *P. inkoi* and *P. parapari*, respectively in the western and the southern sector of the Portuguese continental shelf and the northern limit of *P. guanche* off the south margin of the Nazaré Canyon. *Pisione guanche* is here recorded for the first time in the Lusitanian biogeographic province, increasing to five the number of species known for European continental waters. A taxonomic key for the European *Pisione* species is given.

**Key words:** Iberian Peninsula, distributional range, morphological study, taxonomic key

## Intoduction

The genus *Pisione* Grube, 1857 comprises forty-four species of interstitial polychaetes (Martínez *et al.* 2008; Moreira *et al.* 2010), that can be found world-wide in marine intertidal and shallow subtidal continental shelves; one species lives in freshwater streams of an island on the Pacific of Panamá (San Martín, *et al.* 1998). *Pisione remota* (Southern, 1914) is regarded as being cosmopolitan, and *Pisione puzae* Siewing, 1953 recorded from the Mediterranean*,* were previously the only species recorded from European waters. Recently, *Pisione guanche* San, Martín, López & Núñez, 1999 was described from the Macaronesian biogeographic province (Canary Islands), *Pisione parapari* Moreira, Quintas & Troncoso, 2000 from the Ensenada de Baiona (Galicia, northwestern Spain) and *Pisione inkoi* Martínez, Aguirrezabalaga & Adarraga, 2008 from the Bay of Biscay (northern Spain). Currently, only *P. remota* was recorded from the Portuguese continental shelf, namely in estuaries (Rodrigues & Quintino 1993, 2001; Chainho *et al*. 2008; Rodrigues *et al*. 2011), coastal lagoons (Quintino *et al*. 1987; Carvalho *et al*. 2011) and the near shore shelf (Quintino *et al*. 2001; Freitas *et al*. 2003, 2011; Silva *et al*. 2008).

Wiklund et al. (2005), based on a molecular and morphological analysis, proposed the inclusion of the genus *Pisione* in the family Sigalionidae; however, the family Pisionidae is accepted as a valid family in the majority of publications, so we prefer a more conservative position for the moment and consider it as a separate family of polychaetes.

 *Accepted by P. Hutchings: 26 Jul. 2012; published: 4 Sept. 2012*

Following on from a recent benthic survey undertaken on the Portuguese coastal shelf, this study reports the first record of *P. guanche*, *P. inkoi* and *P. parapari*, and provides a morphological description of each of these species, discusses habitat preferences and biogeographic issues related to their distribution range and presents a key to European *Pisione* species.

## Material and methods

This study is based on more than two hundred and sixty samples obtained between 2007 and 2010 along the entire Portuguese continental shelf (41°51'–37°6'N, 9°1'–7°24'W; Table 1). In the southeastern near shore shelf, the samples were obtained with a Van Veen grab (0.05 $m^2$); all other samples were collected with a Smith-McIntyre grab (0.10 $m^2$). The sediment samples were washed onboard through a 1.0 mm mesh sieve and fixed with 4% formalin neutralized with borax. After hand sorting and taxonomic identification, the animals were transferred for long-term storage in 70% ethanol. The analysis of sediment grain-size and total organic matter content (TOM) followed Quintino et al. (1989). Sediments were classified according to the median value of the particles size diameter using the Wentworth (1922) scale as follows: medium sand (0.25–0.5 mm), coarse sand (0.5–1.0 mm), very coarse sand (1–2 mm) and fine gravel (2–4 mm). The terminology fines, sand and gravel was used to express the amount of particles below 0.063 mm, between 0.063 and 2 mm and above 2 mm respectively. Biogenic content was expressed as the percent ratio between the dry weight of faunal skeletal remains above 2.0 mm diameter size and the total sediment dry weight.

A total of 75 specimens were used for a detailed morphological study of the four *Pisione* species. This includes the measurement of total length, the width at chaetiger 10 (W10), the number of parapodia in complete specimens, the buccal aciculae shape, the length of the dorsal cirrus of parapodia 2 (CP2) and 3 (CP3), the shape of dorsal cirri, the number of teeth of the supra-acicular chaetae (nrT), the protruding length of the notoaciculae through the parapodia (P1), the number and shape of the various types of compound chaetae, the length of the longest blade, the presence/absence of infra-acicular simple chaetae (IA), the number, location and shape of genital organs and the size of female gametes. A multivariate analysis of the morphological data was performed on the basis of a subset of the above descriptors, including W10, P1/W10, CP2/CP3, nrT and IA. This data matrix was submitted to agglomerative hierarchical clustering, using the unweighted pair-group mean average algorithm (UPGMA) and ordination analysis, using Principal Coordinates Analysis, upon the Euclidean distance matrix between specimens, following the variables normalization. The multivariate data analyses were performed with the software PRIMER v.6 (Clarke & Gorley 2006). Significant differences between species were tested using the PERMANOVA+ add-on, permutational multivariate analysis of variance (Anderson *et al*. 2008).

A set of specimens were deposited in the Museu Nacional de História Natural e da Ciência, Lisbon (MB). Additional material remains in the Department of Biology, University of Aveiro, Portugal.

## Results

Table 1 shows the geographical coordinates, depth and sedimentary characterization of the sites where the specimens were collected. Table 2 reports the mean and associated variability of the morphological descriptors studied in the four *Pisione* species and a summary of the environmental characteristics of the sampling sites is presented in Table 3. The specimens are characteristic of coarser sediments as shown by the high gravel and sand content and low fines content. A total of 692 *Pisione* specimens were recorded at 48 sites, belonging to *P. remota* (382 specimens, at 33 sites), *P. parapari* (295, at 35 sites), *P. inkoi* (7, at 4 sites) and *P. guanche* (8, at 6 sites). The present work shows that the four species can co-occur (Fig. 1). Also, *P. remota* was found together with *P. parapari* at 16 sites, *P. remota, P. parapari and P. guanche* at 5 sites and *P. remota* with *P. inkoi* at 1 site. Nevertheless, *P. parapari* (13 sites, mainly in the southern near shore shelf), *P. remota* (10 sites mainly in the western shelf) and *P. inkoi* (2 sites of the western deeper shelf) may occur individually. These *Pisione* species occur in biocoenosis with the molluscs *Thracia villosiuscula* (MacGillivray, 1827)*, Angulus pygmaeus* (Lovén, 1846)*, Caecum* sp.*, Limatula subovata* (Monterosato, 1875)*, Digitaria digitaria* (Linnaeus, 1758), *Gari costulata* (Turton, 1822) and *Goodallia triangularis* (Montagu, 1803), the polychaetes *Protodorvillea keferstein* (McIntosh, 1869) and *Gyptis propinqua*

Marion & Bobretzky, 1875 and the sipunculid *Aspidosiphon (Aspidosiphon) muelleri muelleri* Diesing, 1851 among other species.

**TABLE 1.** Characterization of the sites where *Pisione* specimens were obtained for morphological studies. Gravel = grain-size fraction > 2 mm; Sand = grain-size fraction 0.063–2 mm; Fines = grain-size fraction < 0.063mm; TOM = total organic matter content; NW = northwestern Portuguese continental shelf; W = western Portuguese continental shelf; SW = southwestern Portuguese continental shelf; S = southern Portuguese continental shelf; ST = shelf off Tagus Estuary (Lisbon); MS = medium sand; CS = coarse sand; VCS = very coarse sand; G = fine gravel; "—" = no available data.

| Site | Latitude (ºN) | Longitude (ºW) | Location | Depth (m) | Sediment type | Gravel (%) | Sand (%) | Fines (%) | TOM (%) |
|---|---|---|---|---|---|---|---|---|---|
| R17 | 41°19.477' N | 8°47.210' W | NW | 33.2 | CS | 0.1 | 99.4 | 0.6 | 1.8 |
| R28 | 41°03.434' N | 8°50.691' W | NW | 48.4 | CS | 6.6 | 92.9 | 0.5 | 2.6 |
| R39 | 40°47.302' N | 8°48.870' W | NW | 29.3 | CS | 23.7 | 76.0 | 0.0 | 4.3 |
| R40 | 40°47.426' N | 8°55.030' W | NW | 45.2 | G | 58.4 | 41.5 | 0.0 | 5.4 |
| R46 | 40°39.194' N | 9°02.236' W | NW | 74.1 | VCS | 38.1 | 61.4 | 0.5 | 1.8 |
| R48 | 40°39.295' N | 8°51.291' W | NW | 35.1 | G | 52.9 | 46.9 | 0.1 | 9.9 |
| R51 | 40°30.935' N | 8°56.227' W | NW | 48.5 | VCS | 18.7 | 81.4 | 0.0 | 1.5 |
| R52 | 40°30.983' N | 9°03.664' W | NW | 73.8 | VCS | 37.4 | 61.6 | 0.5 | 0.4 |
| R59 | 40°22.834' N | 8°58.110' W | NW | 54.9 | VCS | 14.0 | 85.6 | 0.4 | 6.7 |
| R68 | 40°06.836' N | 8°59.926' W | NW | 42.3 | G | 62.4 | 37.3 | 0.0 | 1.8 |
| R70 | 39°58.672' N | 9°01.059' W | NW | 38.7 | VCS | 42.2 | 57.1 | 0.6 | 0.2 |
| PC77 | 39°50.732' N | 9°05.010' W | W | 48.2 | G | 50.0 | 49.5 | 0.5 | 0.5 |
| PC85 | 39°26.552' N | 9°16.839' W | W | 44.5 | VCS | 0.2 | 99.4 | 0.3 | 22.9 |
| PC87 | 39°26.671' N | 9°30.526' W | W | 65.6 | VCS | 13.1 | 86.4 | 0.5 | 8.1 |
| PC89 | 39°18.295' N | 9°43.135' W | W | 99.41 | MS | 0.0 | 91.7 | 8.2 | 4.7 |
| PC90 | 39°18.249' N | 9°36.115' W | W | 80.2 | G | 71.2 | 28.6 | 0.2 | 1.3 |
| PC91 | 39°18.361' N | 9°27.269' W | W | 49.8 | VCS | 3.7 | 94.8 | 1.3 | 1.6 |
| PC92 | 39°18.502' N | 9°24.120' W | W | 32.7 | G | 73.1 | 25.8 | 1.1 | 12.4 |
| PC104 | 38°54.414' N | 9°52.202' W | W | 126.9 | — | — | — | — | — |
| PC132 | 38°22.081' N | 8°49.961' W | SW | 25.1 | VCS | 16.0 | 83.7 | 0.1 | 5.8 |
| PC133 | 38°20.696' N | 8°51.550' W | SW | 48.7 | MS | 8.3 | 45.9 | 45.6 | 4.1 |
| PC137 | 38°15.263' N | 8°49.134' W | SW | 37.5 | CS | 17.2 | 82.2 | 0.5 | 3.8 |
| PC138 | 38°09.334' N | 8°49.563' W | SW | 41.0 | VCS | 39.9 | 59.9 | 0.2 | 0.4 |
| PC210 | 36°59.964' N | 8°04.242' W | S | 25.0 | VCS | 32.4 | 63.8 | 3.8 | 17.0 |
| PC222 | 37°05.891' N | 7°34.186' W | S | 15.8 | CS | 4.4 | 94.2 | 1.2 | 10.5 |
| PC229 | 37°06.909' N | 7°24.120' W | S | 13.8 | CS | 3.2 | 93.8 | 2.8 | 14.9 |
| G13(1) | 38°40.863' N | 9°29.006' W | ST | 38.5 | MS | 0.4 | 99.1 | 0.5 | — |
| G21(1) | 38°41.318' N | 9°29.856' W | ST | 43.5 | CS | 7.3 | 92.3 | 0.4 | — |
| ALG2 | 36°57.452' N | 7°52.022' W | S | 9.6 | MS | 0.1 | 100.0 | 0.0 | 0.0 |
| ALG9 | 36°59.066' N | 7°50.234' W | S | 2.6 | CS | 0.1 | 99.7 | 0.2 | 0.5 |
| ALG18 | 36°59.384' N | 7°48.086' W | S | 6.1 | CS | 1.6 | 98.3 | 0.1 | 0.1 |
| ALG19 | 36°59.158' N | 7°48.094' W | S | 9.2 | CS | 2.0 | 97.9 | 0.1 | 0.4 |
| ALG30 | 37°02.081' N | 7°44.036' W | S | 8.7 | CS | 1.6 | 98.0 | 0.4 | 0.6 |
| ALG34 | 37°02.824' N | 7°43.024' W | S | 9.1 | CS | 6.2 | 93.1 | 0.6 | 0.5 |
| ALG39 | 37°02.734' N | 7°42.751' W | S | 9.7 | CS | 0.2 | 99.4 | 0.5 | 3.0 |

*.....continued on next page*

**TABLE 1.** (continued)

| Site | Latitude (ºN) | Longitude (ºW) | Location | Depth (m) | Sediment type | Gravel (%) | Sand (%) | Fines (%) | TOM (%) |
|---|---|---|---|---|---|---|---|---|---|
| ALG42 | 37°03.516' N | 7°41.040' W | S | 13.0 | MS | 0.0 | 99.8 | 0.3 | 5.9 |
| ALG43 | 37°02.941' N | 7°41.031' W | S | 18.1 | CS | 0.4 | 98.9 | 0.4 | 7.5 |
| ALG48 | 37°04.781' N | 7°39.000' W | S | 13.0 | CS | 1.1 | 98.6 | 0.1 | 9.2 |
| ALG49 | 37°05.091' N | 7°38.715' W | S | 13.7 | CS | 0.3 | 98.5 | 1.3 | 8.6 |
| ALG51 | 37°05.556' N | 7°38.027' W | S | 12.3 | CS | 2.2 | 97.4 | 0.4 | 5.6 |
| ALG59 | 37°06.447' N | 7°36.005' W | S | 15.4 | CS | 8.3 | 90.7 | 1.1 | 7.1 |
| ALG60 | 37°05.868' N | 7°35.971' W | S | 16.9 | CS | 10.2 | 89.1 | 0.6 | 5.0 |
| ALG62 | 37°07.811' N | 7°35.047' W | S | 6.5 | VCS | 2.0 | 96.1 | 2.5 | 38.8 |
| ALG63 | 37°06.289' N | 7°35.016' W | S | 16.8 | CS | 15.0 | 84.7 | 0.8 | 9.3 |
| ALG66 | 37°08.003' N | 7°34.020' W | S | 9.3 | CS | 0.3 | 99.4 | 0.3 | 4.4 |
| ALG68 | 37°07.972' N | 7°32.980' W | S | 10.7 | CS | 2.6 | 97.7 | 0.0 | 3.8 |
| ALG71 | 37°08.231' N | 7°32.057' W | S | 11.1 | CS | 5.5 | 94.5 | 0.0 | 5.1 |
| ALG74 | 37°09.006' N | 7°31.061' W | S | 10.5 | CS | 0.9 | 98.6 | 0.5 | 4.4 |

**TABLE 2.** Morphological descriptors mean values and standard deviation (SD) of the *Pisione* species found on the Portuguese continental shelf. W10 = width at chaetiger 10; CP2/CP3 = ratio between the length of the dorsal cirri of parapodia 2 (CP2) and parapodia 3 (CP3); NrT = number of teeth of the supra-acicular chaetae; P1/W10 = ratio between protruding length of the notoaciculae through the parapodia (P1) and W10; IA = presence/absence of infra-acicular simple chaeta; * = incomplete specimen.

| | *Pisione guanche* | *Pisione inkoi* | *Pisione parapari* | *Pisione remota* |
|---|---|---|---|---|
| Number of specimens analysed | 8 | 7 | 30 | 30 |
| Total length (mean ± SD, mm) | 13.933±4.631 | 7.900±1.771 | 4.114±1.390 | 6.915±4.576 |
| W10 (mean ± SD, mm) | 0.495±0.142 | 0.409±0.105 | 0.191±0.023 | 0.302±0.077 |
| Number of parapodia | 22*– 47 | 12*– 47 | 26–51 | 26–97 |
| CP2 (mean ± SD, mm) | 0.095±0.016 | 0.044±0.011 | 0.034±0.006 | 0.038±0.009 |
| CP3 (mean ± SD, mm) | 0.035±0.005 | 0.044±0.011 | 0.024±0.004 | 0.037±0.009 |
| CP2/CP3 (mean ± SD, mm) | 2.693±0.375 | 0.993±0.012 | 1.382±0.081 | 1.008±0.029 |
| P1 (mean ± SD, mm) | 0.032±0.009 | 0.089±0.011 | 0.000±0.000 | 0.000±0.000 |
| P1/W10 (mean ± SD, mm) | 0.066±0.013 | 0.233±0.062 | 0.000±0.000 | 0.000±0.000 |
| Longest blade (mean ± SD, mm) | 0.023±0.002 | 0.050±0.007 | 0.024±0.002 | 0.017±0.002 |
| NrT (number) | 2 | 1 | 2 | 1 |
| IA (number) | 0 | 0 | 0 | 1 |
| Male copulatory organs (chaetigers) | 33–38; 34–41; 40–47 | No male found | 10, 18; 11, 18; 11, 19; 11, 20; 10, 12, 20; 11, 12, 20; 11, 12, 21 | 16–17; 16–18; 20–21; 24–28; 28–35; 29–32 |

## Systematics

### Class Polychaeta Grube, 1850

### Order Phyllodocida Levinsen, 1883

### Family Pisionidae Southern, 1914

**Genus *Pisione* Grube, 1857**

***Pisione guanche* San Martín, López & Núñez, 1999**

**Material examined.** MB29-000239, 1 specimen, site PC137; MB29-000240, 1 specimen, site PC138 (Table 1). Additional material: 1 specimen, site PC90; 1 specimen, site PC92; 1 specimen, site PC132; 1 specimen, site PC133; 2 specimens, site PC138 (Table 1).

**Brief description.** Body width (10$^{th}$ chaetiger) between 0.30 and 0.68 mm, total length between 9.80 and 20.40 mm and total number of chaetigers up to 74. Buccal acicula protruding obliquely, not exceeding chaetiger 1 backwards, with distal margin showing few irregular dentations at tip. All prechaetal lobes bilobed. Dorsal cirri of chaetiger 2, 2.3 to 3.3 times longer than dorsal cirri of chaetiger 3 and subsequent ones (Table 2). All dorsal cirri globular, with a short, spherical distal papilla with a pilose tip, except that of chaetiger 2, which is digitiform. Two types of chaetae: four short-bladed compound heterogomph chaetae (blade length ranging from 18 to 26 μm; cf. Table 2) and one supra-acicular simple chaeta distally bidentate. One stout protruding notoaciculum, up to 0.049 mm. Male genitalia with 6 to 8 pairs of consecutive copulatory organs, in chaetigers 33–38, 34–41, 40–47 (MB29-000240; cf. Table 2). Female sexual organs not visible externally (MB29-000239). Pygidium with two long anal cirri.

**Distribution and habitat.** This species occurred in fine gravel (33%), very coarse (33%), coarse (17%) and medium sand (17%), with low total organic matter content, usually below 1% of total sediment dry weight and high biogenic content (4.6% in average). Specimens were recorded mainly between 25 and 80 m deep (cf. Table 3). This work extends the distribution depth range of this species, previously recorded between 8 and 45 m (San Martín *et al*. 1999). The northern distribution limit of *P. guanche* is now extended to off Peniche (south of the Nazaré Canyon; Fig. 1), being also present immediately south of the Setúbal Canyon, along the southwestern coastal shelf sector. This is the first record of *P. guanche* in the Lusitanian biogeographic province, increasing to five the number of species known for the European continental waters. *Pisione guanche* is presently known from three biogeographic provinces: the Macaronesia (San Martín *et al.* 1999; Moreira *et al.* 2010), the Lusitanian (this study) and the Mediterranean Sea (Çinar 2009 reported it as an alien species on the southern coast of Turkey).

**Remarks.** Dorsal cirri of chaetiger 2 is up to 3.3 times longer than the dorsal cirri of chaetiger 3 whilst San Martín *et al.* (1999) and Moreira *et al.* (2010) reported that the dorsal cirri of chaetiger 2 was only twice as long.

***Pisione inkoi* Martínez, Aguirrezabalaga & Adarraga, 2008**

**Material examined.** MB29-000241, 1 specimen, site PC89; MB29-000242, 1 specimen, site PC104 (Table 1). Additional material: 1 specimen, site R46; 2 specimens, site PC89; 2 specimens, site PC90 (Table 1).

**Brief description.** Width of chaetiger 10 ranging from 0.25 and 0.62 mm, total length from 5.50 to 9.60 mm, with up to 47 chaetigers. Buccal aciculae protruding obliquely, not exceeding length of chaetiger 1, with subrounded distal margin. Prechaetal lobes bilobed in anterior chaetigers and entire on remaining segments. All dorsal cirri globular, small and similar in size (Table 2). Three types of chaetae: three long-bladed compound chaetae (blade length ranging from 39 to 65 μm; cf. Table 2), one short-bladed compound falciger and one supra-acicular simple chaeta, distally unidentate. One stout protruding notoacicula, up to 0.11 mm. Female genital chaetigers with a simple cirriform process developed ventrally at base of parapodium. Female gametes globular, with a diameter ranging from 40 μm to 89 μm, located in chaetigers 29–42 (MB29-000241). No males were found in our samples. Pygidium with two long anal cirri.

**Distribution and habitat.** This species occurred in fine gravel (33%), very coarse (33%), and medium sand (33%), with moderate total organic matter content, 2.4% of total sediment dry weight in average and low biogenic content (1.4% in average). Specimens of this species were recorded mainly in the western Portuguese coast, between 74 and 127 m deep (cf. Table 3). The present study extends the distribution depth range of *P. inkoi*, previously known from 56 to117 m (Martínez *et al*. 2008). This species was known for the northern Iberian Peninsula and this study extends its southern limit to the western sector of the Portuguese continental shelf (Fig. 1).

### *Pisione parapari* Moreira, Quintas & Troncoso, 2000

**Material examined.** MB29-000243, 1 specimen, site PC91; MB29-000244, 1 specimen, site PC132 (Table 1). Additional material: 3 specimens, site R28; 13 specimens, site R39; 6 specimens, site R40; 9 specimens, site R48; 1 specimen, site PC87; 1 specimen, site PC90; 42 specimens, site PC91; 1 specimen, site PC92; 109 specimens, site PC132; 8 specimens, site PC133; 1 specimen, site PC137; 3 specimens, site PC138; 1 specimen, site PC222; 2 specimens, site PC229; 1 specimen, site G13(2); 3 specimens, site G21(1); 4 specimens, site ALG2; 14 specimens, site ALG9; 2 specimens, site ALG18; 6 specimens, site ALG19; 1 specimen, site ALG30; 1 specimen, site ALG34; 16 specimens, site ALG39; 1 specimen, site ALG42; 1 specimen, site ALG43; 3 specimens, site ALG49; 2 specimens, site ALG51; 3 specimens, site ALG59; 4 specimens, site ALG60; 1 specimen, site ALG62; 5 specimens, site ALG63; 10 specimens, site ALG66; 11 specimens, site ALG68; 2 specimens, site ALG71; 1 specimen, site ALG74 (Table 1).

**Brief description.** Width of 10$^{th}$ chaetiger from 0.14 to 0.24 mm, total length from 2.24 to 7.60 mm, and total number of chaetigers ranging from 26 to 51. Buccal aciculae protrude obliquely the skin, not exceeding length of chaetiger 1, with a smooth distal margin. Prechaetal lobes entire. Dorsal cirri of chaetiger 2 is 1.3 to 1.6 times longer than the dorsal cirri of chaetiger 3 and following ones (cf. Table 2). All dorsal cirri globular-piriform, with a papilla with pilose tip, except on chaetiger 2 digitiform. Three types of chaetae: one long-bladed compound heterogomph chaeta with curved tip (blade length ranging from 21 to 28 µm; cf. Table 2), three short-bladed compound heterogomph chaetae and one supra-acicular simple chaeta distally bidentate. One stout notoaciculum embedded in all parapodia. Male genitalia with 2 to 3 pairs of copulatory organs appearing on chaetigers 11, 12, 21 (MB29-000243) or alternating on chaetigers 10, 18, 19, 20 in additional material (cf. Table 2). Female sexual organs not visible externally. Female gametes globular, with a diameter ranging from 38 µm to 64 µm, 49 µm in average, located in chaetigers 20–43 (MB29-000244) or earlier in additional material (19–28), depending on size of specimen. Pygidium with two long anal cirri.

**Distribution and habitat.** This species occurred in fine gravel (11%), very coarse (14%), coarse (63%) and medium sand (11%), with low total organic matter content, usually below 1% of total sediment dry weight and high biogenic content (5.9% in average). Specimens were recorded mainly in the near shore shelf of the western and southern Portuguese coast, between 3 and 80 m water depth, 24.6 m on average (cf. Table 3). The distribution depth range of this species is expanded since *P. parapari* was only previously recorded from 8 to 12 m (Moreira *et al.* 2000). This species was only known in the northern Iberian Peninsula and this study extends its southern limit to the southern sector of the Portuguese continental shelf (Fig. 1).

**Remarks.** In males, copulatory organs usually appeared in alternate parapodia, but in some cases they appeared in consecutive parapodia, which is not consistent with Moreira *et al.* (2000).

### *Pisione remota* (Southern, 1914)

**Material examined.** MB29-000245, 1 specimen, site R70; MB29-000246, 1 specimen, site PC91 (Table 1). Additional material: 2 specimens, site R17; 14 specimens, site R28; 2 specimens, site R39; 13 specimens, site R40; 1 specimen, site R46; 4 specimens, site R48; 25 specimens, R51; 2 specimens, site R52; 15 specimens, site R59; 6 specimens, site R68; 13 specimens, site R70; 4 specimens, site PC77; 55 specimens, site PC85; 12 specimens, site PC87; 7 specimens, site PC90; 30 specimens, site PC91; 21 specimens, site PC92; 42 specimens, site PC132; 7 specimens, site PC133; 23 specimens, site PC137; 5 specimens, site PC138; 2 specimens, site PC210; 2 specimens, site PC222; 55 specimens, site G21(1); 2 specimens, site ALG9; 1 specimen, site ALG39; 1 specimen, site ALG48; 1 specimen, site ALG49; 1 specimen, site ALG59; 1 specimen, site ALG60; 3 specimens, site ALG62; 1 specimen, site ALG63; 4 specimens, site ALG68 (Table 1).

**Brief description.** Width of 10$^{th}$ chaetiger ranged between 0.19 and 0.46 mm, total length up to 25.5 mm and maximum of 97 chaetigers. Buccal aciculae well developed and protruding, with slightly constricted distal ends subdistally and subrounded distal margin. Prechaetal lobe bilobed in anterior parapodia and entire in posterior ones. Dorsal cirri of chaetiger 2 similar to others in size and shape, bulbous with terminal papillae, ranging from 0.022 to 0.060 mm (cf. Table 2). Three types of chaetae: three short-bladed compound chaetae (longest blade up to 21 µm; cf. Table 2), one supra-acicular simple chaeta distally unidentate and one infra-acicular simple chaeta. One stout notoaciculum embedded in all parapodia. Male genitalia with 2 to 8 pairs of consecutive copulatory organs appear between chaetigers 29 and 32 (MB29-000246) or earlier in smaller specimens (16–17; cf. Table 2). Female

genital chaetigers with a simple cirriform process developed ventrally at base of parapodium. Female gametes globular, with diameter ranging from 38 µm to 86 µm, 58 µm in average, located in chaetigers 29–74 (MB29-000245), earlier in smaller specimens (16–36). Pygidium with two long anal cirri.

**Distribution and habitat.** This species occurred mainly in coarse (42%) and very coarse (36%) sand and fine gravel (18%), with low total organic matter content, usually below 1% of total sediment dry weight and high biogenic content (6.6% in average; cf. Table 3). The species was recorded between 3 and 80 m depth, along the western and southern Portuguese coast (Fig. 1). It is widely distributed along the North Atlantic, Mediterranean and Caribbean, at shelf depths (e.g. Dauvin *et al.* 2003; Lourido *et al.* 2010). A detailed revision of specimens from these other areas should be undertaken to confirm its cosmopolitan status (San Martín, 2004).

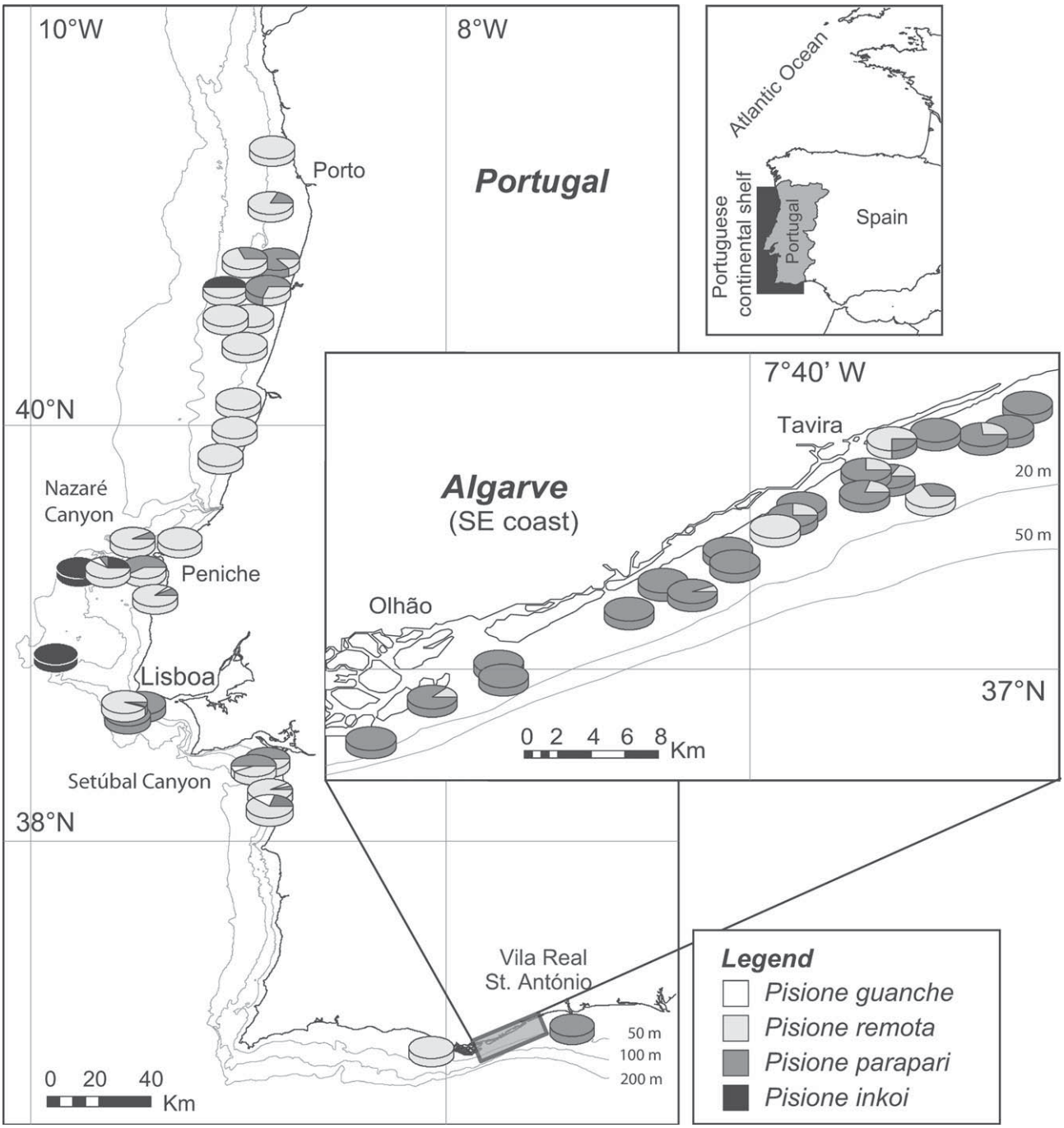


**FIGURE 1.** Distribution and relative abundance of *Pisione* species along the Portuguese continental shelf (northeastern Atlantic).

**Remarks.** In males, the number of pairs of successive copulatory organs varied between 2 and 8 which differed from the literature (4–18; San Martín, 2004). The distribution of female gametes was clearly related to body size, in agreement with Alikunhi (1951).

**TABLE 3.** Environmental characterization of the sites where *Pisione* species occurred along the Portuguese continental shelf (SD = standard deviation). Gravel = grain-size fraction > 2 mm; Sand = grain-size fraction 0.063 –2 mm; Fines = grain-size fraction < 0.063mm; Biogenic fraction = faunal skeletal remains > 2.0 mm; TOM = total organic matter content; MS = medium sand; CS = coarse sand; VCS = very coarse sand; G = fine gravel.

| | *Pisione guanche* | *Pisione inkoi* | *Pisione parapari* | *Pisione remota* |
|---|---|---|---|---|
| Total abundance | 7 | 8 | 295 | 382 |
| Depth (range, m) | 25–80 | 74–127 | 3–80 | 3–80 |
| Depth (mean ± SD, m) | 44.2±16.3 | 95.2±20.6 | 24.6±18.6 | 35.9±20.1 |
| Gravel content (mean ± SD, %) | 37.6±24.3 | 36.4±29.1 | 13.1±20.1 | 22.2±22.4 |
| Sand content (mean ± SD, %) | 54.3±21.4 | 60.6±25.8 | 85.0±21.1 | 75.8±22.8 |
| Fines content (mean ± SD, %) | 8.0±15.6 | 3.0±3.7 | 1.9±7.5 | 2.0±7.8 |
| Biogenic content (mean ± SD, %) | 4.6±3.6 | 1.4±0.3 | 5.9±6.9 | 6.6±7.7 |
| TOM content (mean ± SD, %) | 0.8±0.5 | 2.4±1.7 | 0.9±0.5 | 0.8±0.4 |
| Main sediment types | G (33%), VCS (33%), CS (17%), MS (3%) | G (34%), VCS (33%), MS (33%) | CS (63%), VCS (14%), G (11%), MS (11%) | CS (42%), VCS (36%), G (18%), MS (3%) |

## Multivariate analysis

The classification and ordination analysis based on selected morphological descriptors of *Pisione* species showed a clear separation of the four species (Fig. 2). Axis 1 accounted for 46.6% of the total variation. On the positive pole, this axis separated the species with a proportionally longer dorsal cirri of chaetiger 2 and bidentate supra-acicular chaetae. On the negative pole, axis 1 separated the species with single unidentate supra-acicular chaeta, dorsal cirri of proportional similar length and an infra-acicular simple chaeta. This axis separated *P. guanche* and *P. parapari* on the positive pole, from *P. inkoi* and especially *P. remota* on the negative pole, this latter species being the single one with an infra-acicular simple chaeta. Axis 2 accounted for 31.5% of the total variation and showed the separation of species with higher width of the 10$^{th}$ chaetiger (W10) and with the highest ratio of the protruding length of the notoacicula and W10 on the positive pole (*P. inkoi*), from species with a lower W10 and aciculae embedded throughout the body (*P. parapari* and *P. remota*).

The null hypothesis of no significant differences between the four species, on the basis of the selected morphological descriptors, was rejected with a very large value of the pseudo-F statistic ($p < 0.0001$), shown in Table 4. Such strong rejection of the null hypothesis was due to a much larger sum of squares due to the species than the residual sum of squares, indicating that the intraspecific variability was much lower than the interspecific variability. A similar conclusion can be drawn from the inspection of the mean Euclidean distance within species and between species (cf. Table 4). The highest mean Euclidean distance within species was obtained with *P. guanche* due to the high variability of the W10 values among the specimens of this species. *Pisione parapari* presented the lowest mean Euclidean distance within species denoting a reduced intra species variability regarding the analyzed morphological descriptors. All pairwise comparisons between individual species also rejected the null hypothesis at $p < 0.0001$. Overall, the results showed that the interspecific variability was much higher than the intraspecific variability, supporting the validity of the four Iberian species of *Pisione*. The following key to the *Pisione* species of European waters is based on the understanding gained from the multivariate analysis:

**TABLE 4.** Results of PERMANOVA main test and mean Euclidean distance between and within species.

| Source | df | SS | MS | Pseudo-F | p |
|---|---|---|---|---|---|
| Species | 3 | 332.09 | 110.70 | 207.33 | 0.0001 |
| Residual | 71 | 37.908 | 0.54 | | |
| Total | 74 | 370 | | | |

| | *Pisione parapari* | *Pisione remota* | *Pisione inkoi* | *Pisione guanche* |
|---|---|---|---|---|
| *Pisione parapari* | 0.29 | | | |
| *Pisione remota* | 3.12 | 0.72 | | |
| *Pisione inkoi* | 4.40 | 4.11 | 1.61 | |
| *Pisione guanche* | 3.70 | 4.83 | 4.80 | 1.86 |

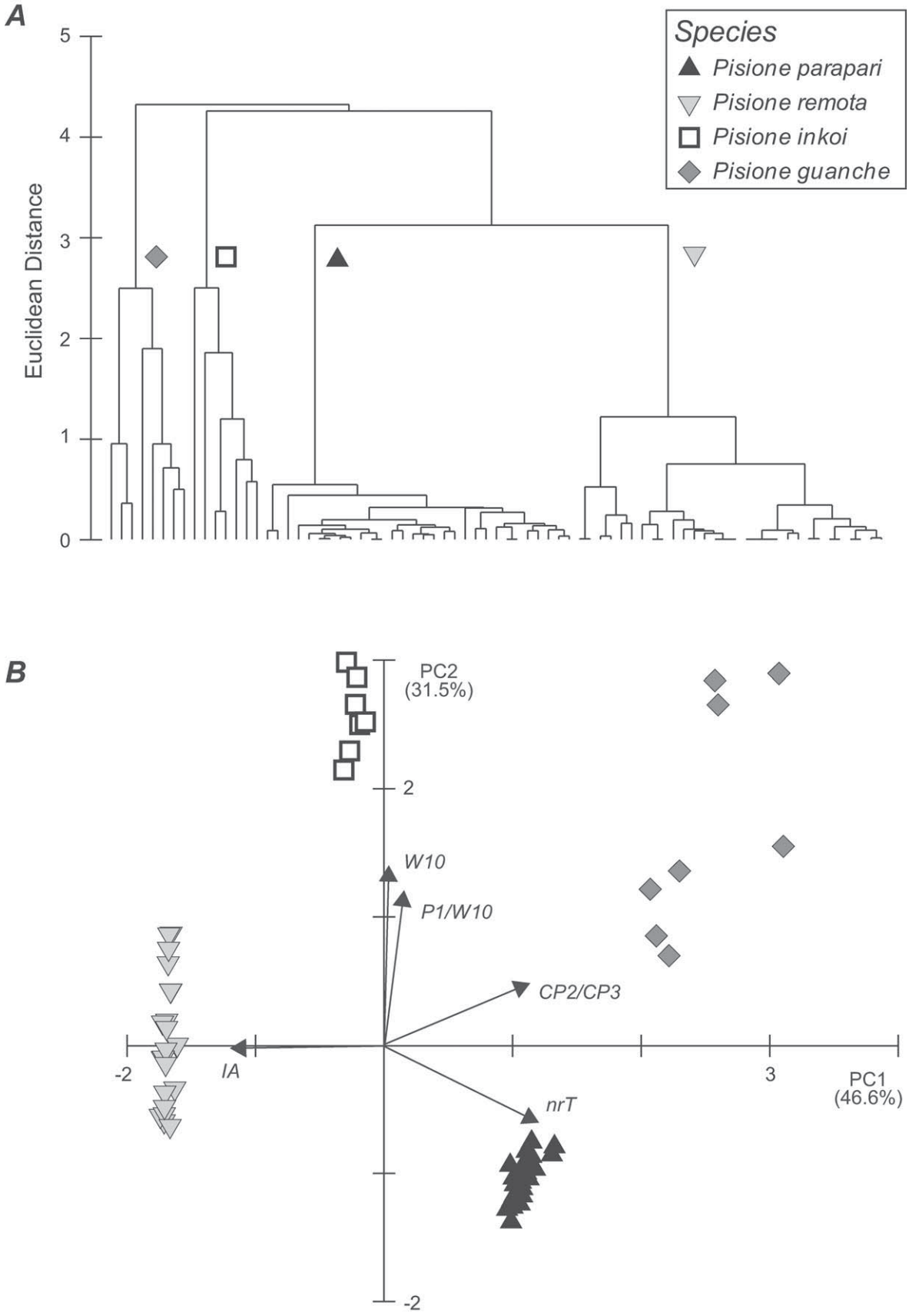


**FIGURE 2.** Classification (A) and Principal Coordinates Analysis (B) based on morphological descriptors of the *Pisione* species occurring in the Portuguese continental shelf. Descriptors are represented as vectors. W10—width at chaetiger 10; CP2/CP3—ratio between the length of the dorsal cirri of parapodia 2 (CP2) and parapodia 3 (CP3); nrT—number of teeth of the supra-acicular chaetae; P1—protruding length of the notoaciculae; IA—presence/absence of infra-acicular simple chaetae.

## Key to the European species of *Pisione*

1. Dorsal cirri of chaetiger 2 up to 3 times longer than dorsal cirri of chaetiger 3, supra-acicular chaetae bidentate . . . . . 2
-. Dorsal cirri of chaetigers 2 and 3 of the same length, supra-acicular chaetae unidentate. . . . . 3
2. Notoacicula protruding from parapodial lobe, male copulatory organs appear in successive parapodia, dorsal cirri in chaetiger 2 from 2.3 to 3.3 times longer than in chaetiger 3 . . . . . *P. guanche* San Martín, López & Núñez, 1999
-. Notoacicula not protruding from parapodial lobe, parapodia bearing male copulatory organs alternate with parapodia without these organs, dorsal cirri in chaetiger 2 from 1.3 to 1.6 times longer than in chaetiger 3 . . . . . *P. parapari* Moreira, Quintas & Trancoso, 2000
3. Notoacicula protruding from parapodial lobe . . . . . *P. inkoi* Martínez, Aguirrezabalaga & Adarraga, 2008
-. Notoacicula not protruding from parapodial lobe . . . . . 4
4. With infra-acicular simple chaetae, prechaetal lobe bilobed in anterior parapodia, jaws without an inward projection . . . . . *P. remota* (Southern, 1914)
-. Without infra-acicular simple chaetae, prechaetal lobes entire, jaws with an inward projection . . . . . *P. puzae* Siewing, 1953*

* Not found in Iberian waters.

In conclusion, the co-occurrence of the four *Pisione* species along the Portuguese continental shelf, some characteristic from Northern Europe and others from Macaronesia, shows the specificity of the Portuguese coast as a region of contact between warmer waters from Northern Africa and the Mediterranean Sea and colder waters from the North Atlantic.

## Acknowledgements

The first author benefited from a PhD grant (SFRH/BD/44231/2008) awarded by the Portuguese Foundation for Science and Technology (FCT—Fundação para a Ciência e Tecnologia) and co-financed by FSE (Fundo Social Europeu). This investigation was supported by the research projects "ACOSHELF" (POCI/MAR/56441/2004—PPCDT/MAR/56441/2004), "Monitorização Ambiental do Emissário Submarino e da ETAR da Guia do Sistema de Saneamento da Costa do Estoril", funded by SANEST, S.A. and "MeshAtlantic" (with the support of the European Union ERDF-Atlantic Area Program 2009-1/110). Colleagues from the Laboratory of Ecology and Biodiversity helped in the sampling and sorting of the material. Authors acknowledge the valuable comments from the referees and the Editor.

## References

Alikunhi, K.H. (1951) On the reproductive organs of Pisione remota (Southern), together with a review of the Family Pisionidae (Polychaeta). *Proceedings: Plant Sciences,* 33(1), 14–31.

Anderson, M.J., Gorley, R.N. & Clarke, K.R. (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. University of Auckland and PRIMER-E, Plymouth. 214 pp.

Carvalho, S., Pereira, P., Pereira, F., Pablo, H., Vale, C. & Gaspar, M. (2011) Factors structuring temporal and spatial dynamics of macrobenthic communities in a eutrophic coastal lagoon (Óbidos Lagoon, Portugal). *Marine Environmental Research,* 71(2), 97–110.

Chainho, P., Chaves, M.L., Costa, J.L., Costa, M.J. & Dauer, D.M. (2008) Use of multimetric indices to classify estuaries with different hydromorphological characteristics and different levels of human pressure. *Marine Pollution Bulletin,* 56, 1128–1137.

Çinar, M.E. (2009) Alien polychaete species (Annelida: Polychaeta) on the southern coast of Turkey (Levantine Sea, eastern Mediterranean), with 13 new records for the Mediterranean Sea. *Journal of Natural History,* 43, 2283–2328.

Dauvin, J.-C., Dewarumez, J.-M. & Gentil, F. (2003) Liste actualisée des espèces d'Annélides Polychètes présentes en Manche [An up to date list of polychaetous annelids from the English Channel]. *Cahiers de Biologie Marine,* 44(1), 67–95.

Freitas, R., Rodrigues, A.M. & Quintino, V. (2003) Benthic biotopes remote sensing using acoustics. *Journal of Experimental Marine Biology and Ecology,* 285–286, 339–353.

Freitas, R., Ricardo, F., Pereira, F., Sampaio, L., Carvalho, S., Gaspar, M., Quintino, V. & Rodrigues, A.M. (2011) Benthic habitat mapping: concerns using a combined approach (acoustic, sediment and biological data). *Estuarine Coastal and Shelf Science,* 92, 4, 598–606.

Lourido, A., Moreira, J. & Troncoso, J. (2010) Spatial distribution of benthic macrofauna in subtidal sediments of the Ría de Aldán (Galicia, northwest Spain). *Scientia Marina,* 74(4), 705–715.

Martínez, J., Aguirrezabalaga, F. & Adarraga, I. (2008) A new species of *Pisione* (Annelida: Polychaeta: Pisionidae) from circalittoral soft bottoms (SE Bay of Biscay, Basque coast). *Cahiers de Biologie Marine,* 49, 283–294.
Moreira, J., Quintas, P. & Troncoso, J.S. (2000) *Pisione parapari* n. sp. a new pisionid from the north-east Atlantic. *Ophelia,* 52, 177–182.
Moreira, J., Veiga, P. & Rubal, M. (2010) First record of *Pisione guanche* (Polychaeta: Pisionidae) at the Azores archipelago. *Marine Biodiversity Records,* 3 e–95, 1–6.
Quintino, V., Gentil, F., Rodrigues, A.M. & Peneda, M.C. (1987) Macrozoobenthic community structure in the lagoon of Albufeira, western coast of Portugal. *Journal of Experimental Marine Biology and Ecology,* 106, 229–241.
Quintino, V., Rodrigues, A. M. & Gentil, F. (1989) Assessment of macrozoobenthic communities in the lagoon of Óbidos, western coast of Portugal. *Scientia Marina,* 53, 645–654.
Quintino, V., Rodrigues, A.M., Ré, A., Pestana, P., Silva, S. & Castro, H. (2001) Sediment alterations in response to marine outfall operation off Lisbon, Portugal: a Sediment Quality Triad study. *Journal of Coastal Research,* SI 34, 535–549.
Rodrigues, A.M. & Quintino, V. (1993) Horizontal biosedimentary gradients across the Sado estuary, Western coast of Portugal. *Netherlands Journal of Aquatic Ecology,* 27, 449–464.
Rodrigues, A.M. & Quintino, V. (2001) Estuarine sediment assessment prior to dredging: integration of benthic and ecotoxicological studies. *Journal of Coastal Research* SI 34, 306–317.
Rodrigues, A.M., Quintino, V., Sampaio, L., Freitas, R. & Neves, R. (2011) Benthic Biodiversity Patterns in Ria de Aveiro, Western Portugal: Environmental-Biological Relationships. *Estuarine Coastal and Shelf Science,* 95, 338–348.
San Martín, G., López, E. & Camacho, I. (1998) First record of a freshwater Pisionidae (Polychaeta): description of a new species from Panama with a key to the species of *Pisione*. *Journal of Natural History,* 32, 1115–1127.
San Martín, G., López, E. & Núñez, J. (1999) Two new species of the genus *Pisione* Grube, 1857 from Cuba and the Canary Islands. *Ophelia*, 51, 29–38.
San Martín, G. (2004) Familia Pisionidae Southern 1914. *In* Ramos, M.A., Alba-Tercedor, J., Belles, X., Gosálbez, J., Geurra, A., MacPherson, E., Serrano, J. & Templado, J. (Eds), *Fauna Ibérica*. Museo Nacional de Ciencias Naturales, CSIC, Madrid, 25, pp. 446–456.
Siewing, R. (1953) Morphologische Untersuchungen am "Kopf" der Pisioniden (*Pisione puzae* nov. spec., Annelida, Polychaeta). *Zoologischer Anzeiger,* 150, 298–313.
Silva, M.C., Pereira, P., Falcão, M. & Fonseca, L.C. (2008) Caracterização das comunidades de anelídeos poliquetas ao longo de um gradiente de profundidade na região do Ancão (Algarve - Portugal). *Pan-American Journal of Aquatic Sciences*, 3(3), 214–231.
Southern, R. (1914) Clare Island Survey. Archiannelida and Polychaeta. *Proceedings of the Royal Irish Academy* 31(47), 1–160.
Wentworth, C.K. (1922) A scale of grade and class terms for clastic sediments. *The Journal of Geology,* 30, 377–392.
Wiklund, H., Nygren, A., Pleijel, F. & Sundberg, P. (2005) Phylogeny of Aphroditiformia (Polychaeta) based on molecular and morphological data. *Molecular Phylogenetics Evolution*, 37, 494–502.